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Modelling clinical data shows active tissue concentration of daclatasvir is 10-fold lower than its plasma concentration

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Objectives: Daclatasvir is a highly potent inhibitor of hepatitis C virus. We estimated the active tissue concentration of daclatasvir *in vivo*.

Methods: We developed a mathematical model incorporating pharmacokinetic/pharmacodynamic and viral dynamics. By fitting the model to clinical data reported previously, we estimated the ratio between plasma drug concentration and active tissue concentration *in vivo*.

Results: The modelling results show that the active tissue concentration of daclatasvir is ~9% of the concentration measured in plasma (95% CI 1%–29%).

Conclusions: Using plasma concentrations as surrogates for clinical recommendations may lead to substantial underestimation of the risk of resistance.

Keywords: hepatitis C virus, resistance, pharmacokinetics/pharmacodynamics, mathematical modeling

Introduction

Daclatasvir is a direct-acting antiviral agent that targets the non-structural protein encoded by the NS5A protein of hepatitis C virus (HCV).[1] It reduces the viral load by 3–4 log in patients after 1–2 days of monotherapy.[2] Combination therapies involving daclatasvir have achieved high cure rates in clinical trials.[3–5] Despite these promising characteristics, resistance can be detected after as few as 3 days of monotherapy.[2] Because direct measurement of drug efficacy in the liver is not feasible, the risk of resistance is often assessed based on the mutant resistance profile measured *in vitro* and the pharmacokinetics of daclatasvir measured in the plasma.[2,6] However, the active tissue concentration, defined as the effective drug concentration acting at the site of infection,[7] is affected by multiple factors *in vivo* and therefore is likely to differ from the plasma concentration. Ignoring this difference may lead to biased conclusions and harmful clinical recommendations.[8] We present a modelling approach to assess this difference based on clinical viral load data and show that the active tissue concentration of daclatasvir is substantially lower than the plasma concentration.

Methods

HCV model and parameter values

We first estimate the efficacy of the drug *in vivo*. Before treatment, the viral population is at a high level at equilibrium. Mathematically, the viral load at the equilibrium, V_0, can be expressed as:[9]

$$V_0 = \frac{p \cdot I_0}{c} \quad (1)$$

where p is the production rate of virions from infected cells, I_0 is the equilibrium level of infected cells before treatment and c is the clearance rate of the virus.

After daclatasvir treatment begins, the HCV viral load declines in several phases.[6,10] After 3–4 days of rapid decline (due to the clearance of free viruses), the viral population enters a quasi-equilibrium where the reduction in viral load is set by the clearance rate of infected cells (assuming the initial baseline strain remains dominant).[10] The viral load during this phase, V^*, can be expressed as $V^*(t) = (1 - \varepsilon_{ave}) \cdot p \cdot I(t)/c$, where ε_{ave} is the

average drug efficacy *in vivo* and $I(t)$ is the abundance of infected cells at time t. In patients treated with a potent inhibitor, such as daclatasvir, the number of newly infected cells during the initial period of treatment is negligible. Hence, the population of infected cells declines exponentially: $I(t) = I_0 \cdot \exp(-\delta \cdot t)$, where δ is the natural death rate of infected cells.[9] Thus:

$$V^*(t) = (1 - \varepsilon_{ave}) \cdot p \cdot I_0 \cdot \frac{\exp(-\delta \cdot t)}{c} \quad (2)$$

Taking the ratio of Equations (1) and (2), we obtain:

$$\varepsilon_{ave} = 1 - \Gamma(t) \cdot \exp(\delta \cdot t) \quad (3)$$

where $\Gamma(t) = V^*(t)/V_0$. Using this equation, we can estimate the value of ε_{ave} from viral load kinetics measured in clinical trials.

Next, we relate the estimated average drug efficacy to the measured pharmacokinetic parameters in the plasma. Here, we assume the active tissue concentration is proportional to the plasma concentration and we define η as the ratio of these two concentrations. The active tissue concentration between doses can be described as:[11]

$$C(t) = \begin{cases} \eta \cdot \left(C_{min} + \dfrac{(C_{max} - C_{min})}{\tau} \right) \cdot t & 0 < t < \tau \\ \eta \cdot C_{max} \cdot \exp(-w \cdot (t - \tau)) & \tau < t < T \end{cases} \quad (4)$$

where τ is the time to reach peak concentration after taking a dose, C_{max} and C_{min} are the peak and trough concentrations measured in plasma, respectively, and T is the interval between doses. We calculate the decay rate of the drug, w, as:

$$w = \frac{1}{T - \tau} \cdot \log\left(\frac{C_{max}}{C_{min}} \right)$$

The drug efficacy over time, $\varepsilon(t)$, is a function of the active tissue concentration, $C(t)$, the cooperativity of the drug, h ($h = 1$ for daclatasvir; H. Qi, C. A. Olson, N. C. Wu, R. Ke, C. Loverdo, J. O. Lloyd-Smith and R. Sun unpublished results), and the half maximal effective concentration of the baseline virus as measured *in vitro*, EC_{50}: $\varepsilon(t) = 1/(1 + (EC_{50}/C(t))^h)$. Adapting earlier results,[11] we calculate the average drug efficacy, ε_{ave}, over a single dosing interval:

$$\varepsilon_{ave} = \frac{1}{T} \int_{t=0}^{T} \frac{1}{1 + EC_{50}/C(t)} dt = \frac{\tau}{T} + \frac{1}{T} \left(\frac{1}{w} - \frac{\tau \cdot EC_{50}}{\eta \cdot (C_{max} - C_{min})} \right) \cdot \log\left(\frac{EC_{50} + \eta \cdot C_{max}}{EC_{50} + \eta \cdot C_{min}} \right) \quad (5)$$

Combining the two expressions of ε_{ave} in Equations (3) and (5), we derive the relationship between the value of EC_{50}, drug pharmacokinetics measured in plasma, and the viral load:

$$1 - \Gamma(t) \cdot \exp(\delta \cdot t) = \frac{\tau}{T} + \frac{1}{T} \left(\frac{1}{w} - \frac{\tau \cdot EC_{50}}{\eta \cdot (C_{max} - C_{min})} \right) \cdot \log\left(\frac{EC_{50} + \eta \cdot C_{max}}{EC_{50} + \eta \cdot C_{min}} \right) \quad (6)$$

This equation enables us to estimate the ratio, η, numerically using pharmacokinetic parameters (including C_{max}, C_{min}, T and τ) reported by Nettles *et al.*[6] and the viral load at day 4 of treatment ($t = 4$) from a subset of patients (Patients E, G, J and N) in Fridell *et al.*[2] (see Table 1 for the values used). We used the viral load at day 4 of treatment in these four patients to ensure that the viral load has reached quasi-equilibrium (>3 days) and that the baseline virus (which is wild-type in these four patients) is still dominant.[10] At timepoints before day 4, the viral dynamics are still dominated by transients and in other patients reported by Fridell *et al.*,[2] resistant mutants have risen to high frequency by day 4; these factors are not considered in the model, so we restrict the data accordingly. Other parameter values used in the estimation are $\delta = 0.14$ day^{-1}, $T = 1$ day and $\tau = 1.5$ h.[6,9] The overall estimate of η is calculated by averaging the values estimated based on data from individual patients.

Uncertainty analysis

To derive the 95% CI for η for each patient, we re-estimated η from 10 000 parameter sets sampled randomly from plausible ranges of values and report the appropriate percentile values. Assumed ranges for the values of EC_{50} and the pharmacokinetic parameters are shown in Table 1; δ is sampled from a triangular distribution from 0.01 to 0.27 with mode at 0.14 day^{-1} [9,12] and τ is sampled from a triangular distribution from 1 to 2 with mode at 1.5 h.[6]

Predicting resistance

We define a mutant as resistant if its reproductive number under drug treatment, $R_{0,drug\_mut}$, is >1, where $R_{0,drug\_mut}$ can be expressed as:

$$R_{0,drug\_mut} = (1 - \varepsilon_{ave\_mut}) \cdot W_{mut} \cdot R_{0,WT} \quad (7)$$

where ε_{ave_mut} is the average efficacy of the drug against that mutant, which can be calculated by substituting the EC_{50} of the

Table 1. Estimated values of η for each patient, and parameter values and data used in the estimation

Patient	Genotype	EC_{50}, nM (SE)	Treatment	C_{max}/C_{min}, nM (SD)[a]	$\text{Log}_{10}\, I^0$[b]	Estimated η (95% CI)
E	1a	0.0059 (0.0038)	10 mg once daily	216/20.4 (88.6/10.0)	−3.2	0.098 (0.02, 0.27)
G	1b	0.0026 (0.0009)	10 mg once daily	216/20.4 (88.6/10.0)	−3.6	0.109 (0.06, 0.25)
J	1a	0.0059 (0.0038)	30 mg once daily	653/55.5 (163/18.9)	−3.1	0.028 (0.01, 0.07)
N	1a	0.0059 (0.0038)	60 mg once daily	1902/176 (247/44.0)	−4.3	0.142 (0.02, 0.31)

Mean of estimated η (95% CI): 0.094 (0.01, 0.29).
[a]Data collected from Nettles *et al.*[6]
[b]Data collected from Fridell *et al.*[2]

Table 2. Comparison of clinical data on resistant mutants and the probabilities of resistance predicted by two models assuming $\eta = 0.094$ or $\eta = 1.0$

Treatment	Dominant resistant mutants observed in the clinical trial reported by Fridell *et al.*[2]	Genotype	Patient(s)	Probability of resistance	
				$\eta = 0.094$	$\eta = 1$
10 mg once daily	**Y93H**[a]	1a	E	**0.684**	**0.236**
	L31V	1a	F	0.999	0.947
	L31M + Y93H	1b	G	0.669	0.499
	L31V + Y93H	1b	G	0.720	0.572
30 mg once daily	Q30E	1a	I, J, K	0.933	0.893
	Y93H[a]	1a	J	**0.556**	**0.021**[b]
60 mg once daily	**Q30H + Y93H**[a]	1a	M	**0.869**	**0.450**
	M28T	1a	N	0.006[b]	0.000[b]
	Q30E	1a	N, O	0.927	0.782
	Q30R	1a	P	0.102	0.000[b]
100 mg once daily	M28T	1a	R	0.0005[b]	0.000[b]
	Q30R + H58D	1a	S	1.00	1.000
	L31V + Q54H + Y93H[a]	1b	T	**0.981**	**0.113**

[a]Bold mutant names denote mutants for which the predicted probabilities of resistance are substantially different between the two models (>0.4 in probability).
[b]Predicted probabilities of resistance <5%.

mutant measured in a replicon system[2] into Equation 5; W_{mut} is the relative fitness of the mutant compared with the wild-type without drug treatment in a replicon system; and $R_{0,WT}$ is the reproductive number for the wild-type virus. We calculated $R_{0,drug\_mut}$ for those mutants that were present at <5% frequency before treatment and rose to become the dominant strain, i.e. with frequency of ≥50%, after treatment as reported by Fridell *et al.*[2] (Table 2). For each mutant, we calculated $R_{0,drug\_mut}$ for 10000 parameter sets sampled randomly. The ranges of variation for the pharmacokinetic parameters, and the values of EC_{50} and W_{mut}, are taken from Fridell *et al.*[2] and Nettles *et al.*;[6] the value of $R_{0,WT}$ is sampled from an asymmetric triangular distribution between 5 and 20, with the mode at 15. The probability of resistance is calculated as the fraction of parameter sets for which $R_{0,drug\_mut} > 1$.

Results and discussion

Based on viral load data from clinical trials and pharmacokinetic parameters from plasma,[2,6] we estimated the ratio of the active tissue concentration to the plasma concentration of daclatasvir, η, to be 0.094 (95% CI 0.01–0.29; Table 1). This estimation is consistent across different dosing regimens (Table 1). Thus, the active tissue concentration of daclatasvir in the liver is much lower than the concentration measured in plasma, contrary to common assumptions.[1,5] To test our method, we used the model to predict resistant mutants *in vivo* based on resistance profiles measured in replicon systems for HCV genotypes 1a and 1b.[2,6] In general, the model predictions agree well with clinical data (Table 2). Almost all mutants that appeared as dominant resistant mutants in clinical trials were correctly predicted by the model, with the exception of M28T (probability of resistance <5%). M28T has a low value of EC_{50} *in vitro*,[2] but it appeared as resistant in two patients treated with 60 mg and 100 mg once daily regimens. This discrepancy may arise from additional differences in the viral genomes or the difference between the replicon system and *in vivo* conditions. Several other mutants were predicted to be resistant, but did not rise to high frequencies in clinical trials. Again, this could arise from artefacts of replicon systems or the mutants could be resistant *in vivo* but remain at low frequencies due to competition from other resistant mutants with higher replicative fitness, such as Q30E in genotype 1a.

If we assumed no difference between the active tissue concentration and the plasma concentration (i.e. assumed $\eta = 1.0$), the model underestimated the resistance potential for numerous mutants (Table 2). Two resistant mutants identified in clinical trials, Y93H and Q30R, were predicted to have very low probabilities of resistance. Also, the model substantially underestimated the probability of resistance for two mutants, Q30H+Y93H and L31V+Q54H+Y93H, compared with predictions assuming $\eta = 0.094$.

Altogether, these results suggest that the active tissue concentration for daclatasvir is ~10-fold lower than its plasma concentration. There are several possible reasons for this low active concentration: (i) the drug may not penetrate well into liver tissue or hepatocytes; (ii) the drug may be bound by proteins or other chemicals in the liver; (iii) the conformation or local environment of NS5A is different, which may reduce the accessibility or affinity to the drug; and (iv) heterogeneities in the distribution of drug and/or virus in the liver may cause infected cells to be exposed to lower drug concentrations.[7] Neglecting this difference can lead to substantial underestimation of the resistance potential of mutant viruses. Another possibility is that the EC_{50} measured *in vitro* differs from the EC_{50} *in vivo*, though this has not been found for other drugs.[13–15] If this was the case, then η can be interpreted as a composite parameter incorporating both the difference in EC_{50}

and the difference in tissue versus plasma concentrations; this does not alter its importance when assessing resistance risk using EC_{50} measured *in vitro* and drug concentration measured in plasma.

Our work highlights the importance of estimating the active therapeutic concentration to make accurate predictions about the resistance profile of a drug. We have used a modelling approach, including uncertainty analyses to account for the challenge that pharmacokinetic data are not available from the same patients as virological data. Future studies would be strengthened by datasets that collect all pertinent information for the same individuals. We believe this method is also applicable to other highly potent antiviral drugs for which the quasi-equilibrium state is reached before resistant strains are selected to significant frequency. However, for certain drug classes, such as drugs that act by blocking viral entry, the approach to quasi-equilibrium may be too slow and alternative methods will be needed to assess active tissue concentrations.

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Transparency declarations

None to declare.

References

1 Gao M, Nettles RE, Belema M *et al*. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010; **465**: 96–100.

2 Fridell RA, Wang C, Sun JH *et al*. Genotypic and phenotypic analysis of variants resistant to hepatitis C virus nonstructural protein 5A replication complex inhibitor BMS-790052 in humans: in vitro and in vivo correlations. *Hepatology* 2011; **54**: 1924–35.

3 Lok AS, Gardiner DF, Lawitz E *et al*. Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012; **366**: 216–24.

4 Pol S, Ghalib RH, Rustgi VK *et al*. Daclatasvir for previously untreated chronic hepatitis C genotype-1 infection: a randomised, parallel-group, double-blind, placebo-controlled, dose-finding, phase 2a trial. *Lancet Infect Dis* 2012; **12**: 671–7.

5 Karino Y, Toyota J, Ikeda K *et al*. Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir. *J Hepatol* 2013; **58**: 646–54.

6 Nettles RE, Gao M, Bifano M *et al*. Multiple ascending dose study of BMS-790052, a nonstructural protein 5A replication complex inhibitor, in patients infected with hepatitis C virus genotype 1. *Hepatology* 2011; **54**: 1956–65.

7 Theuretzbacher U. Tissue penetration of antibacterial agents: how should this be incorporated into pharmacodynamic analyses? *Curr Opin Pharmacol* 2007; **7**: 498–504.

8 Mouton JW, Theuretzbacher U, Craig WA *et al*. Tissue concentrations: do we ever learn? *J Antimicrob Chemother* 2008; **61**: 235–7.

9 Neumann AU, Lam NP, Dahari H *et al*. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-α therapy. *Science* 1998; **282**: 103–7.

10 Guedj J, Dahari H, Rong L *et al*. Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life. *Proc Natl Acad Sci USA* 2013; **110**: 3991–6.

11 Wahl LM, Nowak MA. Adherence and drug resistance: predictions for therapy outcome. *Proc Biol Sci* 2000; **267**: 835–43.

12 Snoeck E, Chanu P, Lavielle M *et al*. A comprehensive hepatitis C viral kinetic model explaining cure. *Clin Pharmacol Ther* 2010; **87**: 706–13.

13 Zhou Y, Bartels DJ, Hanzelka BL *et al*. Phenotypic characterization of resistant Val36 variants of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* 2008; **52**: 110–20.

14 Chu HM, Zhou Y, Bartels DJ *et al*. Telaprevir (VX-950)-resistant variants exhibit reduced replication capacity compared to wild-type HCV in vivo and in vitro. *J Hepatol* 2007; **46**.

15 Adiwijaya BS, Herrmann E, Hare B *et al*. A multi-variant, viral dynamic model of genotype 1 HCV to assess the in vivo evolution of protease-inhibitor resistant variants. *PLoS Comput Biol* 2010; **6**: e1000745.